Further Tests of the Hypothesis That Influenza in Pregnancy Causes Malformations

IAN LECK, M.B., Ph.D.

A RECENT STUDY of malformations recorded on U.S. birth certificates (*1*) suggested that cleft lip and limb deficiencies might be especially common among children born after A2 influenza epidemics. The data for clefts covered the first major epidemics in three States and subsequent outbreaks in two of these States and in an aggregate of 17 Standard Metropolitan Statistical Areas (SMSA's). Each of the three re-exposures, but none of the three initial outbreaks, was associated with a relatively high incidence of cleft lip without cleft palate among infants born 26 to 40 weeks later. A study of incidence in Birmingham, England, following the initial A2 outbreak and three epidemics due to recurrence of the A2 or B strains (*2, 3*) had previously yielded similar findings for all clefts of the lip, but the incidence of cleft lip without cleft palate was not examined separately in this study.

The U.S. birth certificates examined for data on malformations other than clefts covered only one epidemic—the recurrence in the SMSA's. Apart from cleft lip, reduction deformities of the limbs were the only defects that showed a significant increase after this epidemic. The Birmingham reports did not include comparable data on limb deficiencies.

In this paper I have reclassified the Birmingham data on lip and limb defects to make them comparable with those from the United States, and analyzed in the same way more recent data on these and other defects for the whole of England and Wales. I have also used both the British and the U.S. data to determine whether the increases in incidence extended throughout the period from 26 to 40 weeks after each influenza epidemic. If maternal exposure to the influenza virus can affect a process like the formation of the upper lip, the speed at which the embryo develops is likely to limit to a few days or less the period during development when it is susceptible to this effect, in which case the intervals between epidemics and the births of most of those affected would have a much narrower range than 26 to 40 weeks.

Dr. Leck is senior lecturer in community medicine, University College Hospital Medical School, London, England. He has also worked in the department of social medicine, University of Birmingham, England, and in the epidemiology branch, Dental Health Center, National Institutes of Health, Public Health Service, San Francisco, Calif., where some of these data originated. Tearsheet requests to Ian Leck, Community Medicine Section, 115 Gower Street, London WC1E 6AS, England.

Sources of Data

Most of the sources of data have been fully described elsewhere. In Birmingham a variety of methods had been used to ascertain (*a*) malformations of all kinds diagnosed at ages 0 to 6 years in children (including the stillborn) delivered in 1950–54 and at 0 to 2 weeks in infants born in 1955–59 (*4*) and (*b*) selected types of malformations among children born in 1960–65, all of whom were at least 6 months old when ascertainment was finished (*5*). The information about infants born in 1955–59 has since been augmented from records of hospital admissions in 1955–65. As in previous Birmingham studies (*2, 3*), influenza was assumed to have been epidemic when the number of new claims to Sickness Benefit—a social security payment made to those off work for more than 3 days because of illness—filed in the city during each of 2 consecutive weeks or more was at least 1,500 above the 1953–61 average for the time of year. As claims were not enumerated locally before 1953, births in this year and earlier years were excluded from the analysis.

For the whole of England and Wales, statistics of malformed infants (including the stillborn) delivered in 1964–68 were made available by the General Register Office, London (now the Office of Population Censuses and Surveys) to which defects observed in newborn children are reported under a scheme introduced in 1964 (*6*). To estimate the periods when in-

fluenza was epidemic in the related population, the weekly numbers of new claims to Sickness Benefit actually received in England and Wales each year from 1963 to 1968 (*7*) were compared with the numbers that would have been received if the claims for that year had been distributed between weeks in the same proportions as the total for the other 5 years of the period. After inspection of the results and of contemporary virological reports, it was decided to regard influenza as epidemic during the weeks when the actual weekly number of new claims exceeded by 50,000 or more the estimate based on experience in other years.

The U.S. data include abstracts of the birth certificates of children born alive in California, Pennsylvania, and Wisconsin in 1955–61 and in 17 SMSA's of Eastern United States in 1962–65. The related influenza epidemics were identified from records of the National Communicable Disease Center (now the Center for Disease Control) of the Public Health Service (*1*).

Methods of Analysis

As in previous reports, the children whose records were examined for possible effects of maternal exposure to influenza, subsequently called "high risk children," were those in the British series who were born betwen 26 and 40 weeks after epidemics, and the U.S. infants born between the closest approximations to these intervals that were possible with the available data. The possibility that defects might be especially common among the high risk children was

Table 1. Cleft lip and reduction deformities ascertained among all infants, liveborn and stillborn, Birmingham, England, 1954–65

Type of malformation	Births 26–40 weeks after epidemics (high risk)				Other births 1954–65 (low risk)	Crude incidence ratios (Incidence in high risk births of year stated / Incidence in all low risk births)				
	Mar. 12–Aug. 5, 1958	July 29–Dec. 8, 1959	June 21–Oct. 31, 1961	June 20–Oct. 30, 1962		1958	1959	1961	1962	All years
Cleft lip	3	6	6	5	86	0.95	[1] 2.24	1.92	1.55	[1] 1.64
Cleft palate with cleft lip	5	8	12	7	168	.81	1.53	[1] 1.97	1.11	1.35
Reduction deformities of limb(s)	7	5	6	2	116	1.65	1.39	1.43	.46	1.22
Total births [2]	7,961	6,757	7,877	8,158	217,210					

[1] $0.05>\frac{P}{2}>0.01$.

[2] Estimated from monthly totals on the assumption that births each month were evenly spaced within the month.

Table 2. Number of selected malformations reported for all infants, liveborn and stillborn,

Type of malformation	Births in 1966 and 1968 (post epidemic years)				Births in 1964–65 and 1967 (low risk years)		
	a July 27–Dec. 6, 1966 [1]	b Rest of 1966	c June 26–Oct. 22, 1968 [1]	d Rest of 1968	e Total	f July 27–Dec. 6 [2]	g June 26–Oct. 22 [3]
Anencephalus	408	825	361	787	3,926	1,408	1,173
Spina bifida	558	1,058	471	1,064	4,791	1,569	1,406
Encephalocele	42	75	36	86	399	137	110
Cleft lip	135	226	101	203	1,021	330	301
Cleft palate with cleft lip	174	324	173	348	1,475	506	463
Tracheo-esophageal fistula, esophageal atresia, and stenosis	41	84	35	72	363	114	106
Atresia and stenosis of rectum and anal canal	72	123	56	137	557	199	177
Reduction deformities of limb(s)	60	97	55	99	454	160	139
Exomphalos	85	151	79	154	721	252	213
Total births [5]	306,014	557,052	266,521	564,599	2,611,781	916,628	839,479

[1] Post epidemic periods.

[2] Equivalent to high risk period in 1966.

[3] Equivalent to high risk period in 1968.

[4] $0.05>\frac{P}{2}>0.01$.

[5] Estimated from monthly totals on the assumption that each month's births were evenly spaced.

explored by computing two kinds of ratios: the crude incidence ratio (incidence of a defect in a high risk period, divided by its incidence in all low risk births of the same population) and the standardized incidence ratio (the observed incidence in the high risk period divided by an estimate of the incidence to be expected during this season and year, the formula for this estimate being

$$\frac{a\ B}{A}$$

where a is the incidence rate in the low risk seasons of the post epidemic year, A the rate during the same seasons of the years with no high risk period, and B the rate during the rest of these years). A more detailed account of these ratios has been given elsewhere (*1*).

Standardized ratios have the advantage of being much less likely than crude ratios to be biased by the seasonal fluctuations and secular trends that affect the incidence of some malformations. In a relatively small series like the Birmingham one, this advantage is outweighed by the fact that each standardized ratio has a much greater sampling variance than the corresponding crude ratio. For this reason, standardized ratios for the Birmingham data are not given here. The methods used in calculating the other ratios, and in testing significance when ratios above unity occurred, were those described previously (*1*) with the following modifications: (*a*) separate ratios were calculated for four phases of each high risk period (the first 3 months and the remainder) as well as for the whole; and (*b*) for greater convenience, the division of time into post epidemic and low risk years required during the above method of standardization was made in the data for England and Wales at the beginning and end of each calendar year that included a high risk period, whereas in the U.S. studies this had been done 6 months before and 6 months after the midpoint of each high risk period.

Results

In Birmingham, England, only four influenza epidemics (*2, 3*) were identified during the years when children born in 1954–65 were passing through early intrauterine life. The A2 strain had been reported to be prevalent during the first three epidemics, and the B strain during the fourth. The frequency of cleft lip and reduction deformities of the limbs among infants born after these epidemics and at other times is shown in table 1. Cleft lip without cleft palate was significantly more common among all high risk children than others. Cleft lip and palate and reduction deformities showed smaller increases that were not statistically significant. Each increase was associated with three of the four epidemics: the two groups of clefts were not especially common after the initial A2 outbreak and the incidence of reduction deformities did not rise after the epidemic of influenza B.

In England and Wales, as a whole, influenza was estimated to have been at an epidemic level during two periods when children born in 1964–68 were at risk of teratogenesis: January 26 to March 1, 1966, and December 27, 1967, to January 16, 1968. A2 and B strains, mainly B, were isolated during the first outbreak, and A2 during the second. As the births following these two outbreaks had not previously been studied, attention when analyzing them was paid to defects of all nine types suggested by previous work (*1, 2, 8*) to be associated with maternal influenza. The results of this analysis are given in table 2. The only figure significantly in excess of unity was the standardized ratio for cleft lip after the 1966 epidemic, but the corresponding ratio for 1968 was almost as high. The high-

England and Wales, 1964–68

Incidence ratios, 1966 epidemic		Incidence ratios, 1968 epidemic	
Crude	Standardized	Crude	Standardized
0.90	0.87	0.91	1.08
.98	1.07	.95	1.07
.92	1.05	.90	1.10
1.14	**1.23**	.98	1.19
.99	1.01	1.13	1.09
.96	1.05	.94	1.18
1.08	1.04	.96	.88
1.13	1.12	1.19	1.26
1.01	1.03	1.08	1.23

NOTE: Boldface letters relate to incidence ratios at right.

Formulas for the various incidence ratios were as follows:

1966 epidemic:

$$\text{Crude ratio} = \frac{a(B+D+E)}{A(b+d+e)}$$

$$\text{Standardized ratio} = \frac{aBF(e-f)}{Abf(E-F)}$$

1968 epidemic:

$$\text{Crude ratio} = \frac{c(B+D+E)}{C(b+d+e)}$$

$$\text{Standardized ratio} = \frac{cDG(e-g)}{Cdg(E-G)}$$

where A, B...G are the related populations for a, b...g.

est ratios were for reduction deformities after the 1968 outbreak, and except for cleft lip these defects also had the highest ratios after the 1966 epidemic. No other defect showed as much as a 10 percent rise in incidence after each of the two outbreaks.

The incidence rates and ratios of cleft lip and reduction deformities in different phases of the periods following the British and U.S. epidemics when increases occurred are compared in table 3. The basic data used in computing these figures were not included since they would occupy more space than their importance warrants. The increases were not consistently associated with births during any single phase of the periods in question. Only in one case of 11—that of cleft lip after the 1966 epidemic—were there significant differences in incidence between phases. The maximums in four of the six rows of ratios for cleft lip occurred during the first or second month, but the exceptions included the highest ratio of all (in the fourth phase of 1963). With almost equal inconsistency, the highest standardized ratio for reduction deformities (also in 1963) was in the first month, but the maximums in the other three rows of ratios for these defects occurred during the third or fourth phase.

Discussion

The results of the study of British children born 26 to 40 weeks after influenza epidemics (tables 1 and 2) showed several similarities to those previously reported from the United States (*1*). First, cleft lip without cleft palate was more common than expected after each epidemic owing to recurrence of a familiar strain (A2 in 1959 and 1961 and B in 1962 in Birmingham; A2 and B in 1966 and A2 in 1968 in England and Wales) but not after the first A2 epidemic (1957) in Birmingham. Second, each A2 outbreak was associated with an increase of more than 10 percent in the incidence of reduction deformities. And third, the incidence ratios for the other malformations examined (neural tube defects, cleft lip and palate, esophageal and anal atresias, and exomphalos) were well below those for cleft lip alone and limb defects, and low enough to make it reasonably certain that the marked excesses reported from Dublin (*8*) for neural tube defects and from Birmingham (*2*) for atresias and exomphalos were far from typical.

To account for the association between cleft lip and influenza outbreaks due to familiar but not new strains of virus, it was suggested previously that this defect might be caused by something that happens when pregnant women who already have some immunity to a particular strain are reexposed (*1*). If this were so, the risk would probably be greatest when the exposure occurred at a particular stage in development and would fall off rapidly in embryos at earlier and later stages.

Table 3. Frequency of cleft lip and reduction deformities during high risk periods, by month of birth

Type of malformation	Incidence (per 1,000) during high risk periods				Incidence during low risk periods	Incidence ratios[2]			
	1st month[1]	2d month[1]	3d month[1]	Remainder[1]		1st month	2d month	3d month	Remainder
Cleft lip:									
Birmingham, England:[3] Epidemics of 1959, 1961, and 1962	0.81	0.82	0.83	0.61	0.40	2.06	2.07	2.10	1.54
Pennsylvania:[4] Epidemic of 1959	.47	.46	.15	.30	.32	1.99	1.87	.55	1.20
Wisconsin:[4] Epidemic of 1960	.69	.54	.23	.36	.32	1.75	2.06	.85	1.22
17 metropolitan areas:[5] Epidemic of 1963	.29	.19	.30	.43	.25	1.20	1.26	1.09	2.40
England and Wales:[6]									
Epidemic of 1966	[7].61	[7].36	[7].31	[7].47	.39	1.87	1.19	.86	1.12
Epidemic of 1968	.28	.47	.39	.38		.75	1.64	1.43	1.16
Reduction deformities of limb(s):									
Birmingham, England:[3] Epidemics of 1957, 1959, and 1961	.84	.43	1.30	.70	.53	1.58	.80	2.44	1.31
17 Metropolitan areas:[5] Epidemic of 1963	.40	.25	.28	.48	.27	2.20	1.27	1.07	1.90
England and Wales:[6]									
Epidemic of 1966	.20	.14	.19	.23	.17	1.14	.95	1.25	1.12
Epidemic of 1968	.27	.13	.19	.24		1.49	.78	1.25	1.55

[1] Exact dates of birth were not available for all series; therefore, months vary in length between 28 and 31 days.
[2] Incidence ratios for Birmingham are crude; others are standardized.
[3] Live and still births 1954–65.
[4] Live births 1956–61.
[5] Live births 1962–65.
[6] Live and still births 1964–68.
[7] Variations in incidence between different phases of high risk period were significant at the 5 percent level.

As a result, each post epidemic increase in the incidence of cleft lip would be largely confined to only one or two of the four phases into which the period 26 to 40 weeks after each epidemic was divided (table 3). And although these periods differed in duration and in the ways they were divided, the differences were sufficiently small for the greatest increase to have been expected to occur during the same phase of each period, or at least in one or other of a pair of adjacent phases, if the relationship between cleft lip and malformations had been causal. In fact there was no such consistency between series, and the only increase that was concentrated to a significant extent within one phase of a post epidemic period was that observed among children born 182 to 211 days after the onset of the 1966 epidemic in England and Wales. Unless born prematurely, which is not usual in cases of cleft lip alone (*9*), most of these children would have been conceived more than 8 weeks before the outbreak started, by which time their facial clefts should have closed (*10*).

The relationship between reduction deformities and influenza is likewise made more difficult to accept as causal by the increases in their incidence not being concentrated within the same one or two phases after every epidemic.

Other explanations that might be suggested for the behavior of both lip and limb defects are, first, that this may be a result, although an unusual one, of random variation; second, that the proportion of cases of these defects that are reported, but apparently not of others, may increase after epidemics; and, third, that the survival of embryos affected by these defects or exposed to conditions predisposing to them may be differentially affected by epidemics. Although each of these suggestions raises problems, each is easier than the hypothesis of causation to reconcile with the finding that the increases in incidence were not confined to children who had passed through a particular stage of development during the epidemics.

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Statistics concerning children born 26 to 40 weeks after four influenza epidemics in Birmingham, England, between 1957 and 1965 were found to resemble published U.S. data in showing increases in the incidence of reduction deformities of the limbs following outbreaks of A2 influenza and of cleft lip without cleft palate after each epidemic except for the first caused by the A2 strain. In England and Wales, epidemics in both 1966 and 1968 were followed by increases of more than 10 percent in the incidence of defects in these two categories reported to the General Register Office, but the other malformations associated with influenza in previous British studies showed no such increase.

Both the U.S. data and those for Birmingham and for England and Wales as a whole were used to compare the incidence of lip and limb defects in different phases of the periods 26 to 40 weeks after epidemics. The increases in incidence during these periods did not seem to be confined to children who were at any particular stage of development during the epidemics, which suggests that the association between influenza and these defects is unlikely to be causal.